CHIRON | BLOOD TESTING
Trust Through Innovation

Use of Radiolabeled Platelets for Assessment of In Vivo Viability of Platelet Products

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Desirable Platelet Radionuclide Tracer Characteristics

- Objective
  - Internal or external quantitation of platelet kinetics.

- Radionuclide Characteristics
  - Readily detectible.
  - Physiologic element.
  - Non toxic to cell/patient.
  - No perturbation of study.
  - Minimal reutilization/elution.
  - Ease of administration/sampling.
  - Selective tracer uptake.
  - Homogeneous cellular distribution

Radionuclides - Principles & Practice

Principle

Representative Dose	Stable Donor	Defined Distribution	Sample Precision
Harvest Representative Aliquot	Variable Turnover	Estimated Volume	Constant Volume
No Selective Process Loss		Assumes Consistency	Consistent Sample Timing
Consistent Tracer Uptake	Variable Cell Quality	Affirm Steady State	
No Label Damage/Elution			Accurate Counting

Practice

43mL Whole Blood	Time Paired Studies	Nomogram Blood Volume Estimates	Weighed 2mL Samples
10- 20 ml Platelet Concentrate			
Tube Processing >80% recovery	Concurrent Studies	Dilute 3 Standards 1:5000	Correct for Injectate Plasma Elution
Uptake 60- 80% ^{111}In & 20– 40% ^{51}Cr		5 -10% Immediate In-vitro Elution	Use 10 Day RBC Activity Correction
1 X Soft + 2 X Hard Spins		Platelet Counts	Count to 2% Error

^{111}In & ^{51}Cr Labeling Characteristics

	^{111}In -Oxine	^{51}Cr – Sodium Chromate
Desirable Emission	90-94% @ 172 & 247 kev	9% @ 320 kev
Tissue Selective Uptake	Plt >> WBC >>> RBC	RBC >>> Plt >> WBC
Non Toxic (Target/Patient)	Oxine @ 3-6 ug/mL	Chromate < 10^4 molar
Detection Parameters	171 kev → ~72% eff 245 kev → ~53% eff	320 kev → ~3% eff
Elution (RBC & Plt)	8%/day & 11% @ day-1	1-2%/day & 6% @ day-1

^{111}In & ^{51}Cr Tracer Characteristics

	^{111}In - Oxine	^{51}C – Sodium Chromate
Administration Ease	↑ Transferrin Avidity (Wash)	Activity ≥ 20 uCi/ug Cr (Plt Count)
Clearance	Plasma T ½ ~10 Hours	R/E → Excretion @ 3%/day
Reutilization	Nil post oxine ↓	Nil post chromate → Chromic
Cell Uptake	Cells Equivalent (80% cytosol)	Energy Dependent (ATP associated)
Half Life	2.8 days → rapid counting	28 days → delayed counting
Counting Technology	Correct for count times	3” crystal NaI detector=~ 2x Eff.

Detection Implications of ^{111}In & ^{51}Cr Physical Characteristics

- Low ^{51}Cr photon yield mandates high efficiency (NaI) counters.
- Photon scatter requires ^{111}In sum peak counting.
- ^{51}Cr may be counted directly with scatter correction.
- 28 day ^{51}Cr T ½ allows late counting post ^{111}In decay.
- Low dose (low count) infusions need long count times.
- 2.8 day ^{111}In T ½ requires elapsed time count correction.
- Rapid post sample processing and counting is desirable.
- Standard counts should be diluted to ≃ sample counts.

Development of a Double Label $^{111}In/^{51}Cr$ Assay Method

- Purpose
  - Develop comparable ^{111}In & ^{51}Cr platelet techniques for consistent results.
- Study Plan
  - Evaluate relationship between in vivo & in vitro elution.
  - Evaluate ^{111}In labeling effects on platelet function.
  - Assess ^{111}In and ^{51}Cr RBC activity and evolution.
  - Develop corrections to support generation of equivalent outcomes.
- Studies
  - Studies were performed using a similar tube/electrolyte method.
- Procedure Development
  - Develop a procedure for simultaneous ^{111}In and ^{51}Cr platelet labeling.
  - Generate a simultaneous ^{111}In and ^{51}Cr infusion, sampling, and counting procedure.
  - Validate the essential equivalence of the two methods.

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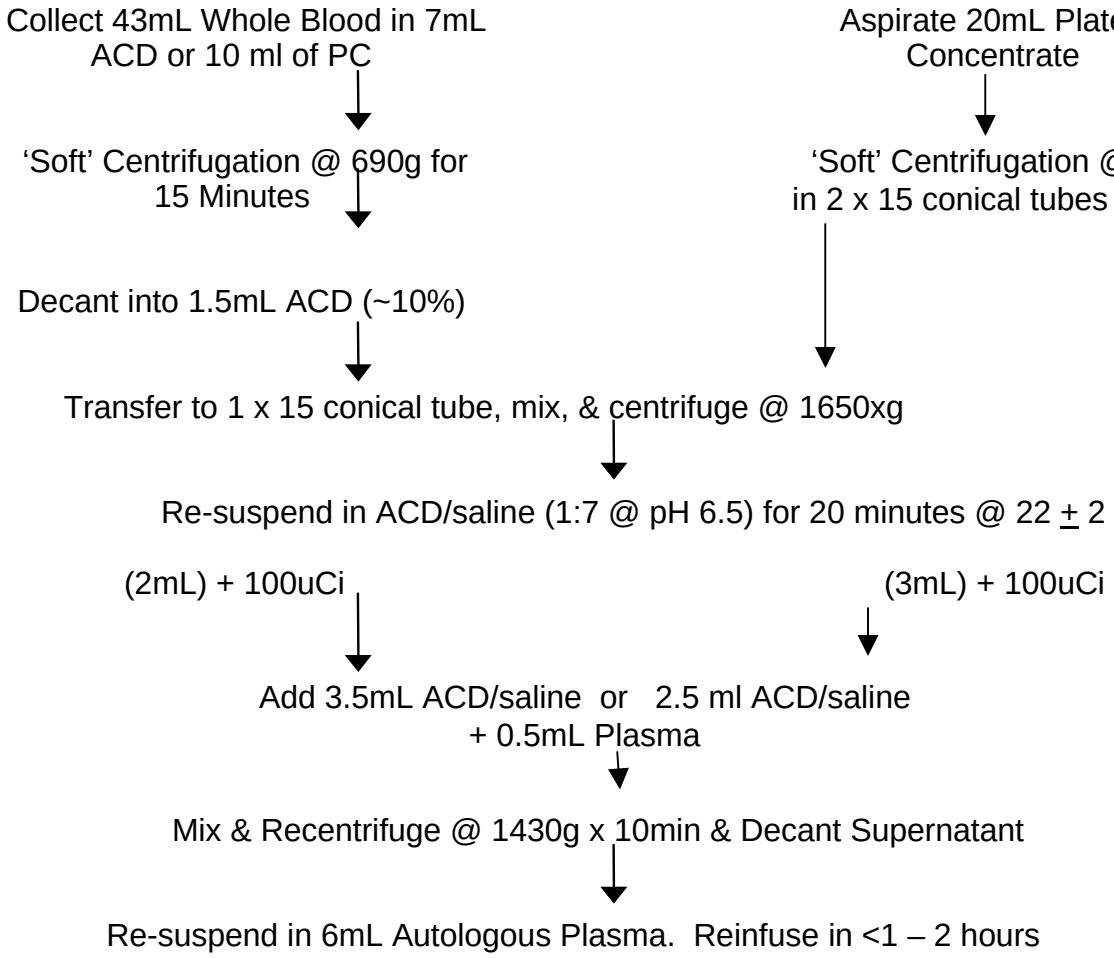
Double Isotope Platelet Procedure Development – Labeling Process



Development of a Double Label $^{111}In/^{51}Cr$ Assay Method

- Elution (A)
  - 63 In vivo/in vitro studies performed.
  - Early injectate, diluted injectate, and in-vitro/in-vivo elution analysis.
  - Injectate processing method developed ⟶ injectate correction.
- BioDistribution & RBC Elution (B)
  - 15 simultaneous ^{111}In and ^{51}Cr imaging and kinetic studies performed.
  - 0, 5, 10 day stored CPD-PC concentrates studies.
  - In vivo whole body and organ uptake measured over 24 hours.
  - RBC activity quantitated over 10 days ⟶ RBC correction.
- $^{111}In/^{51}Cr$ Double Label Validation (C)
  - 16 concurrent ^{111}In & ^{51}Cr 5 day PC storage studies.
  - Post infusion platelet, RBC/WBC, & plasma activity density separated.
  - Double manual apheresis cross over study design developed.
  - Validation study to define sample size requirements.

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Double Label $^{111}In/^{51}Cr$ Development Studies

(Study A) In Vivo Plasma Activity (Mean ± SD)

Label Uptake	72 ± 8%	42 ± 10%
Labeling Loss	35± 9% (similar)	
In-vivo - Post Tx Plasma Activity (%)	^{111}In	^{51}C
5 Minutes	5 ± 1	3 ± 2
1 Hour	6 ± 2	1 ± .5
3 Hours	6 ± 2	0.8 ± 7
In-vitro Plasma Activity		
Neat Injectate (2 hours)	3 ± 3%	6 ± 3%
Diluted Injectate (2 hours)	* 11 + 4%	**9 + 4%

* In Vivo/In Vitro Correlation r = 0.82 * (@ 3 hours) r = 0.77** + (@ 5 minutes)

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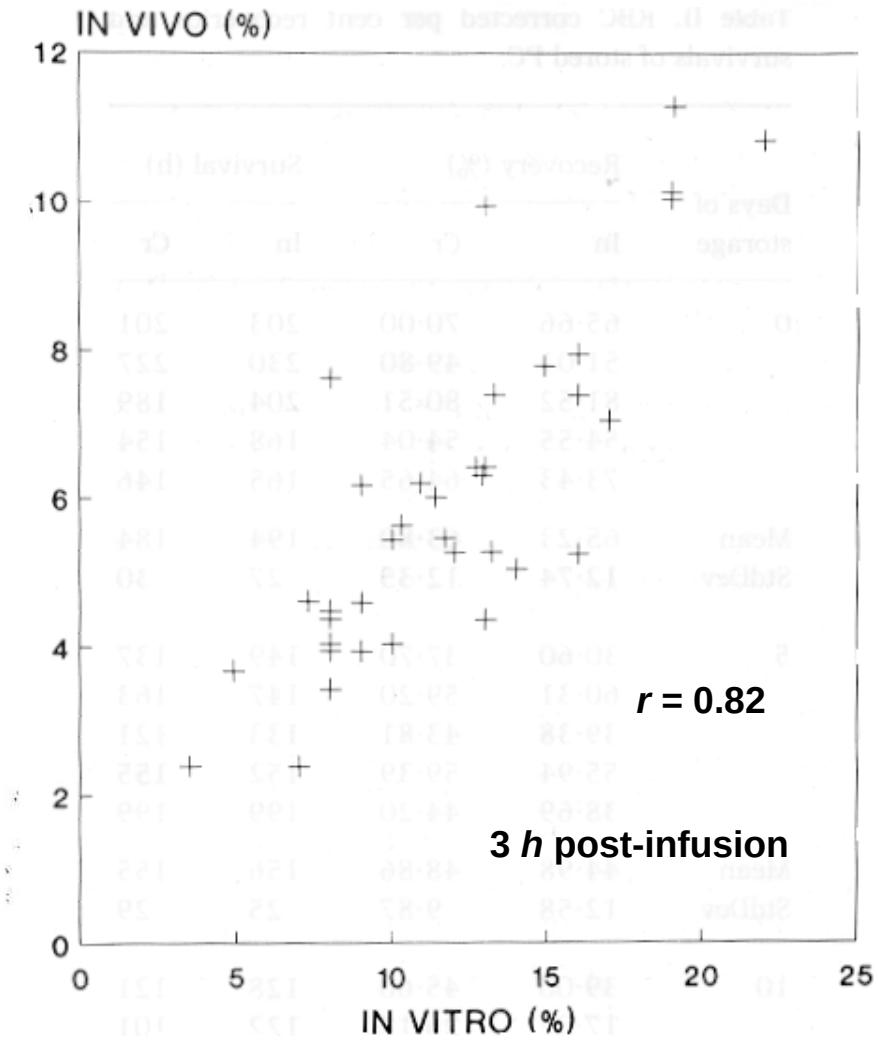
Comparison of In Vitro & In Vivo ^{111}In Activity



In vitro **^{111}In plasma activity of diluted injectate in fresh whole blood and *in vivo* plasma activity.**

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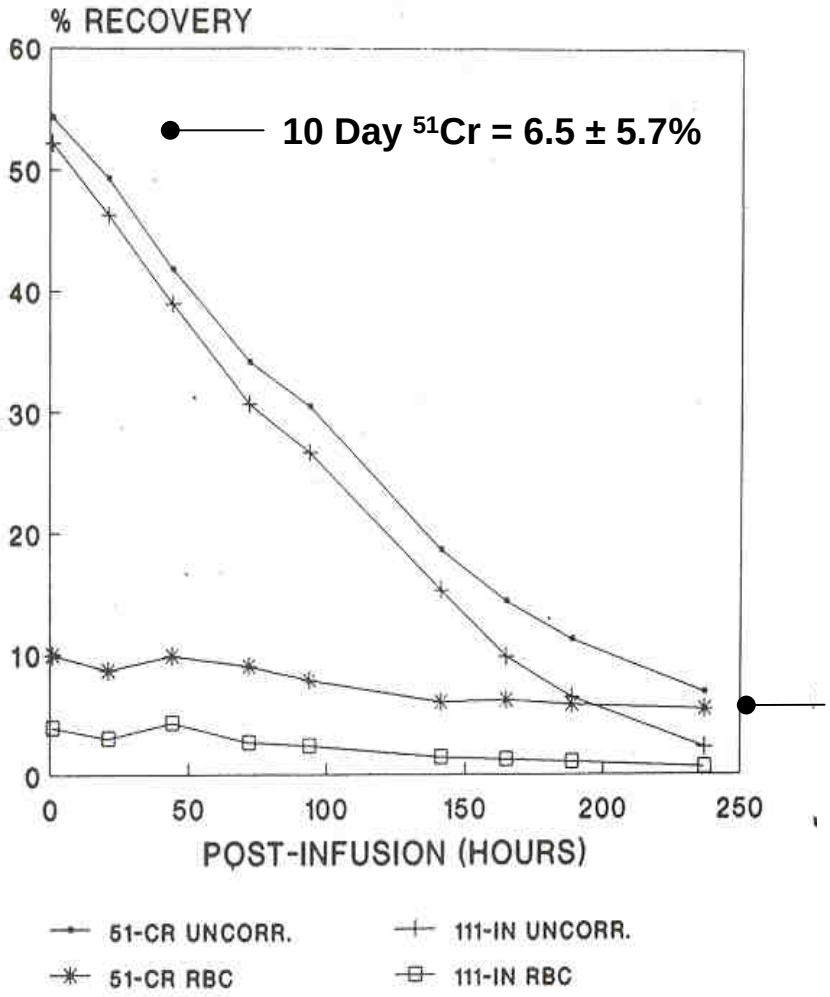
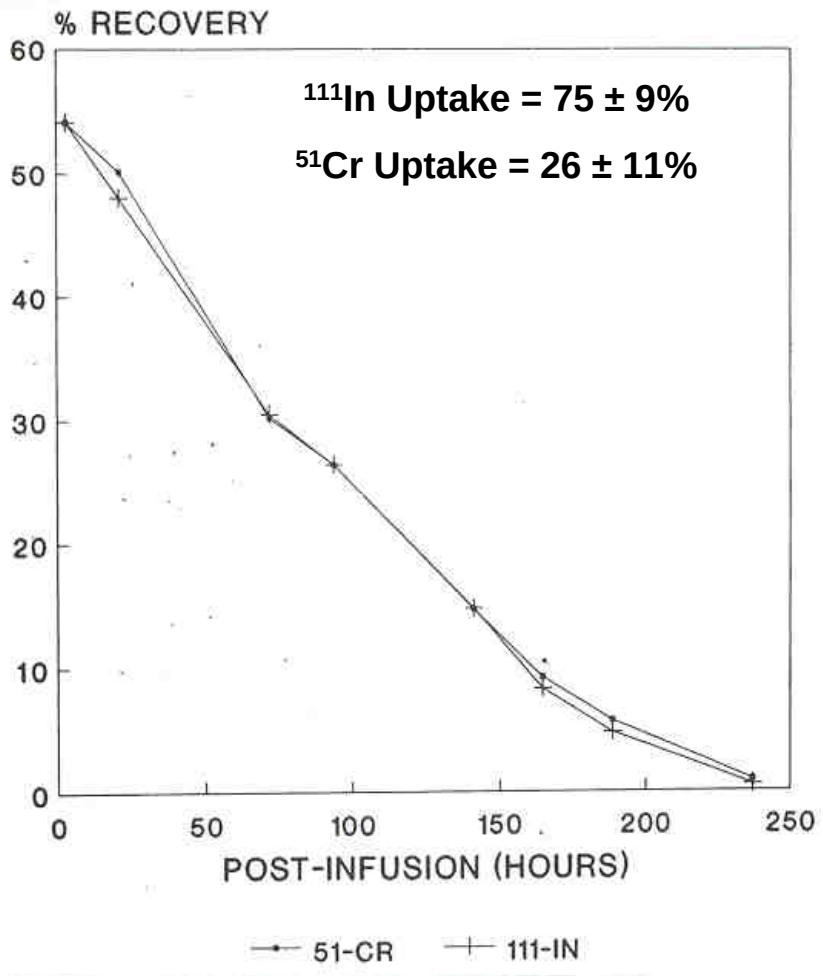
^{51}Cr RBC Uncorrected & Corrected Recoveries





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Double Isotope Platelet Procedure – Elution & Plasma Correction

- Injectate
  - Retain aliquot of ^{111}In-only and ^{51}Cr-only injectate for standards.
  - Mix ~ 15uCi of injectates and retain an aliquot for standards.
  - At the time of infusion, add 10uL injectate to 10mL fresh EDTA blood.
    - Centrifuge after 2 hours @ 37 °C & calculate elution fraction.
  - Prepare 3 x 1 in 5000 individual & mix injectate standards.

- Samples
  - Collect 7mL EDTA samples @ 3 hours, 7 samples/10 days.
    - Prepare 2mL weighed aliquots for WB counting, & centrifuge an additional 2ml for split counting to correct for plasma activity.
  - Count individual, and mix standards; samples; splits to a 2% accuracy.
    - Correct standards for elution and samples for RBC & plasma activity.

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Corrected Post Transfusion ^{111}In & ^{51}Cr Platelet Kinetics

Study B	Storage Duration	^{111}In	^{51}C
% Recovery	0	65 ± 13	64 ± 12
	5	45 ± 13	49 ± 10
	10	24 ± 13	29 ± 12
Survival (Hours)	0	194 ± 27	184 ± 30
	5	156 ± 25	155 ± 29
	10	72 ± 53	63 ± 53
Study (C)			
% Recovery	5	66.1 ± 10.6	65.6 ± 10.9
Survival (hours)	5	164.4 ± 25.5	164.4 ± 31.5
Integral (% hours)	5	6026 ± 1185	5958 ± 1240

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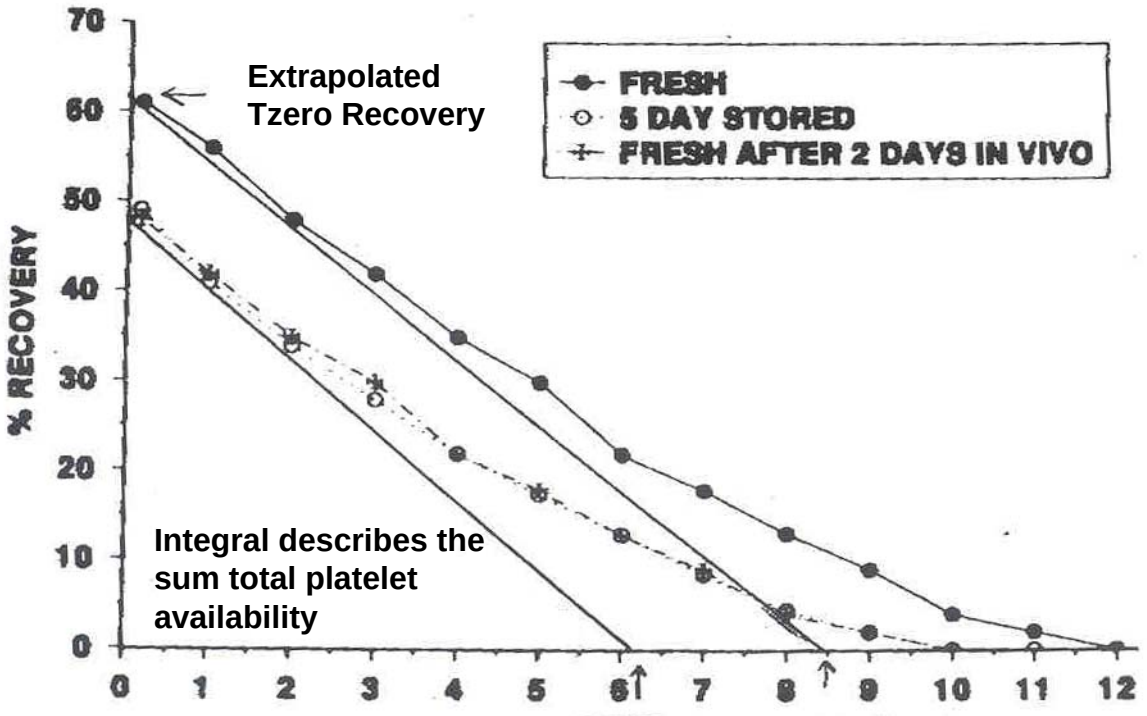
Platelet In Vivo Kinetic Calculation Principles



Survival in Days = Numerical Expected Lifespan

BJH 91:212:1995

Sample Size – Concurrent vs. Separate

% Recovery (absolute %)

Detection Goal	10%	7.5%	5%
Separate	16	22	32
Concurrent	5	6	8

Survival – (Hours)

Detection Goal	30 Hours	25 Hours	20 Hours
Separate	16	22	32
Concurrent	5	6	8

Table shows sample size required to detect a listed difference with 80% power and with alpha = 0.05.

Storage Duration & ^{111}In Platelet Kinetics

- Purpose
  - Evaluation of in vivo kinetics, storage duration, and in vitro parameters.
- Design
  - 35 time separated paired ^{111}In platelet kinetic studies.
  - Test (PAS) and control (CPDA-1) P.C. stored from 0.5 to 10 days @ 22 °C.
  - ^{111}In studies performed with plasma correction.
  - Post transfusion recoveries (PTR), survival (numerical expected lifespan), and integral (area under curve) were quantitated.
  - Degree of exponential (random loss vs. senesce) estimated.
  - Relationship between in vivo and in vitro parameters compared.
  - In vitro measures included pH, HSR, ESC, ATP & lactate production.

Vox Sang 59:12:1990

Storage Duration & ^{111}In Platelet Kinetics

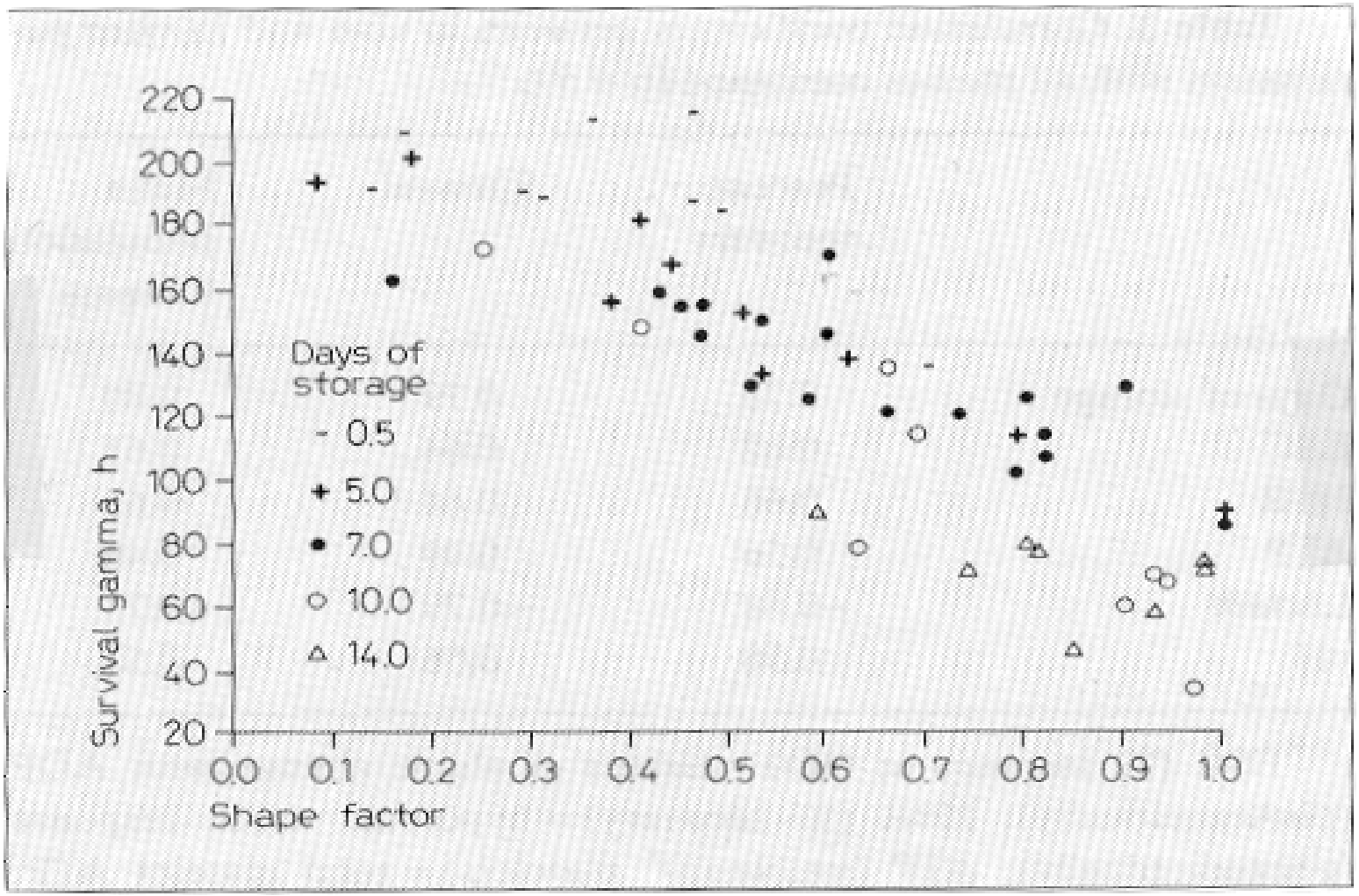
Days Stored	% Recovery	Survival (Hours)	Shape Factor
0.5	55 ± 10	189 ± 24	.38 ± .17
5	41 ± 11	146 ± 41	.55 ± .30
7	37 ± 11	107 ± 39	.83 ± .14
10	23 ± 9	74 ± 43	.87 ± .14
14	9 ± 8	51 ± 24	.88 ± .09

Implications

- Both PTR and survival decreased with storage duration.
- Donor variability mandated double label studies.
- Lactate, morphology, and pH independently correlated with ^{III}In kinetics.

Vox Sang 59:12:1990

Storage Duration & Non Linear Loss



Vox Sang 59:12:1990

Double Label ^{111}In Fresh & ^{51}Cr – Stored Platelet Study Design

- Paired in vivo ^{51}Cr studies were performed ~ 28 days apart.
- 18 whole blood donations were randomly processed into BC-PC or PRP-PC.
- Following 5 days of 22 °C storage, ^{51}Cr in vivo studies performed.
- Concurrent fresh ^{111}In and stored ^{51}Cr studies performed.
- Test vs. control outcomes were compared.
- Stored Cr values were expressed as a % of fresh In values to give Relative Recoveries and Survivals.
- Platelet in-vitro studies included cell counts, pH, O2 & glucose consumption, lactate production, ATP, morphology, HSR, ESC.
- Platelet GP1b and LDH release rates were also measured.

Transfusion 32:113:1992

In Vivo Variables of fresh ^{111}In-labeled & 5-day stored ^{51}Cr-labeled PRP-PC* & BC-PC†

Platelet Recovery (%) ‡

	^{111}In Fresh	^{51}Cr 5-day-stored	^{51}Cr/^{111}In relative (%)
PRP-PC	60 ± 7	49 ± 10	81 ± 9
BC-PC	64 ± 6	53 ± 8	85 ± 10

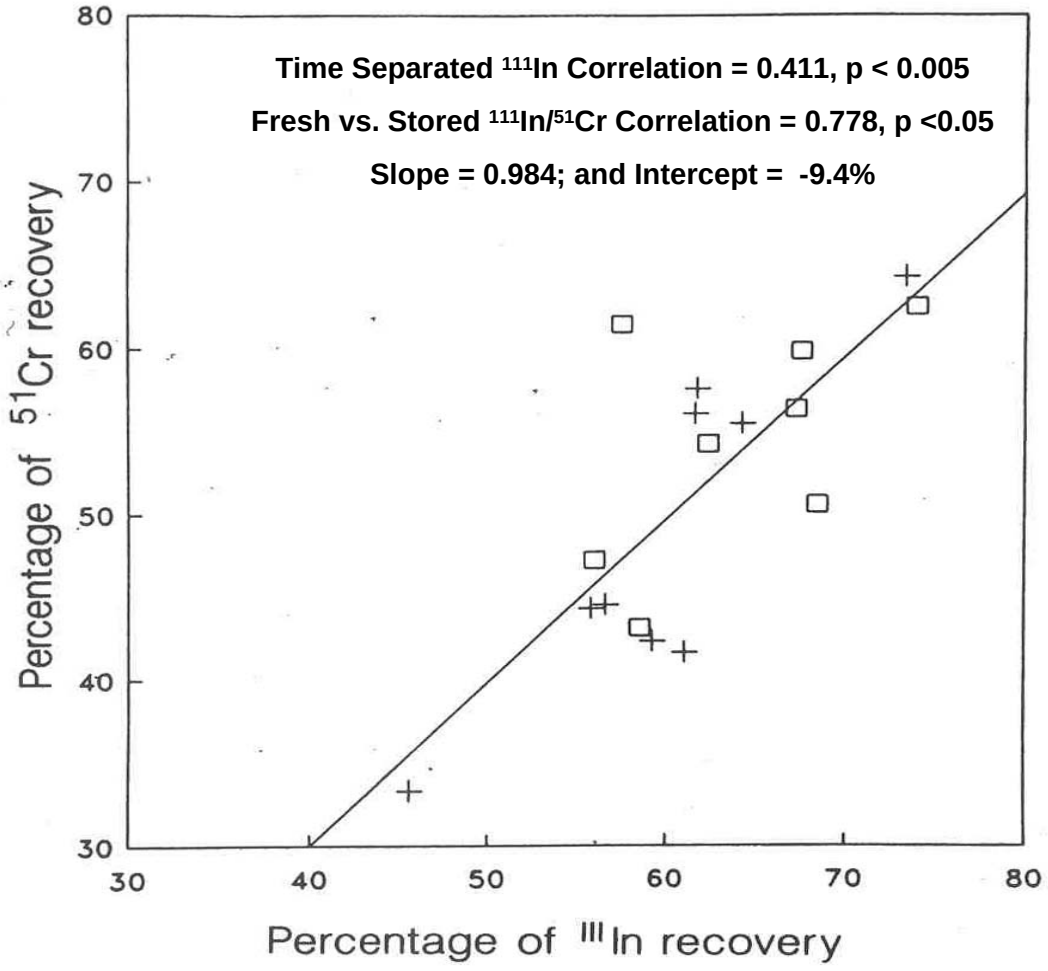
Platelet Survival ‡

	^{111}In Fresh (hrs)	^{51}Cr 5-day-stored (hrs)	^{51}Cr/^{111}In relative (%)
PRP-PC	210 ± 22	162 ± 29	77 ± 10
BC-PC	209 ± 30	163 ± 20	79 ± 11

* Platelet-rich plasma-platelet concentrates.
† Buffy coat-PCs.
‡ Mean ± SD.

Transfusion 32:113:1992

Paired Concurrent Comparison of ^{111}In Fresh & ^{51}Cr Stored PC



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Simultaneous Paired ^{111}In & ^{51} Cr Reduced Platelet Volume Study Design

- Apheresis into standard and reduced volume P.C.
- 20 unit study with randomized order labeling with ^{111}In and ^{51}Cr.
- Simultaneous infusion of ~ 15uC, ^{51}Cr and ^{111}In labeled platelets.
- Elution and red cell correction applied.

- Relative Recoveries (>35mL)
  - % Recoveries = 99 (95 - 103)
  - Integral (% .days) = 99 (96 – 101)

In Vivo Mean (95% CI)

Values	30 – 34mL P.C.	35 – 50mL P.C.	
% Recovery	80 (69-92)	99 (95-103)	p = .005
Survival (days)	89 (83-94)	103 (98-107)	p = .0005
Integral (% days)	81 (69-93)	99 (96-101)	p = .012

Transfusion 34:39:1994

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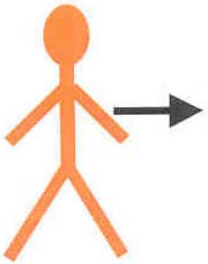
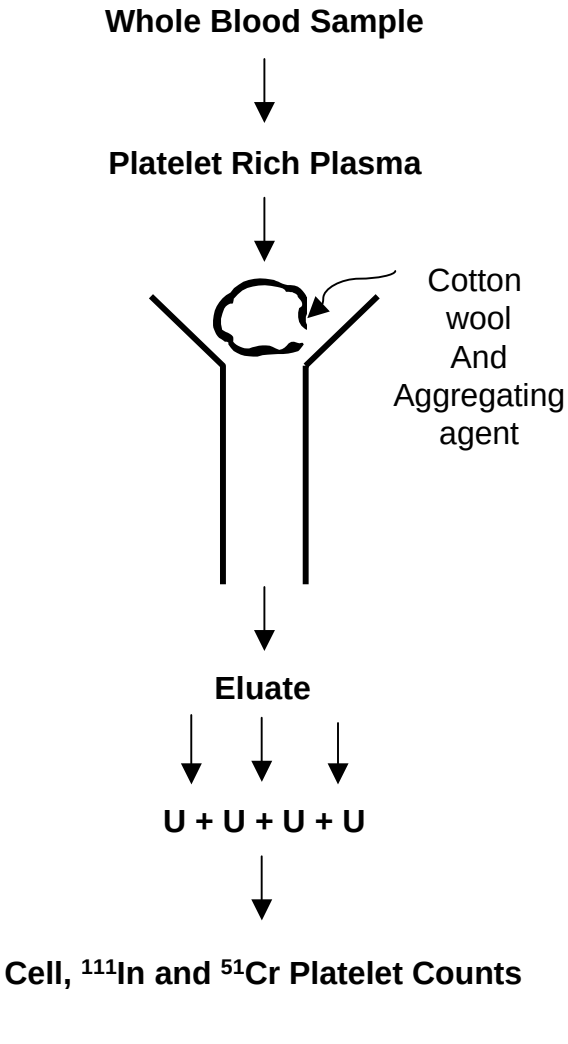
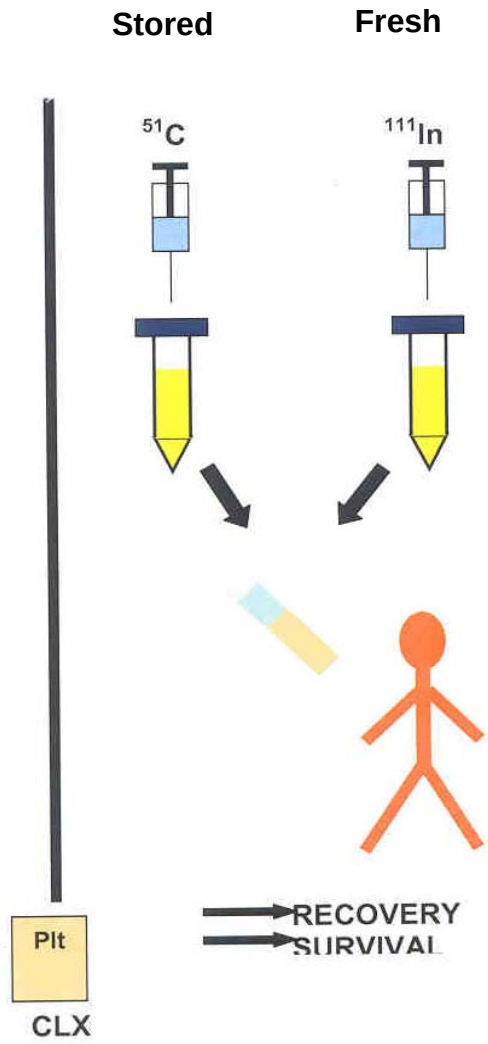
In Vivo Post Transfusion Functional Recovery Study



n = 12





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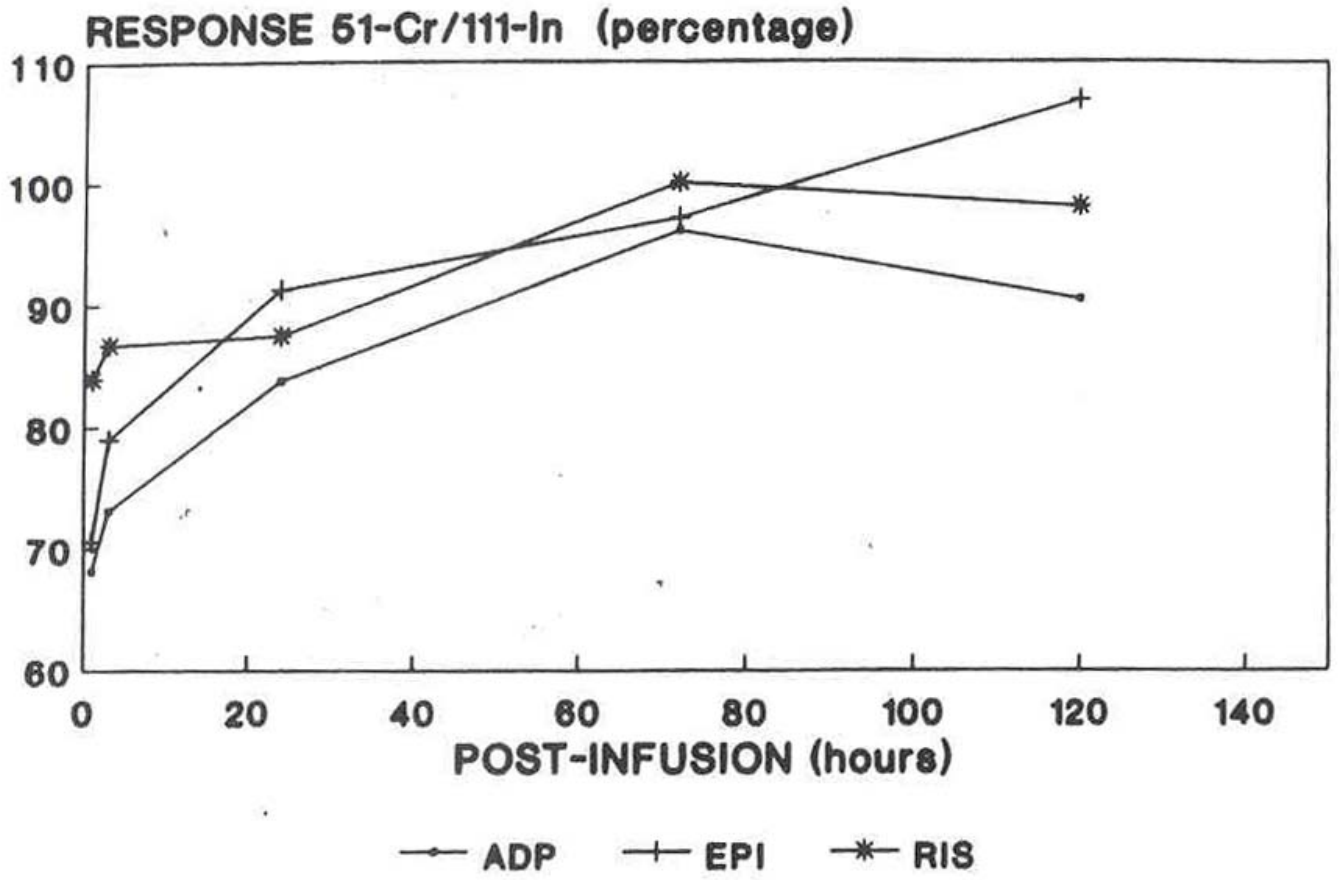
Ex-Vivo Aggregation of ^{51}Cr Stored Platelets



Percent ^{111}In labeled fresh platelet response

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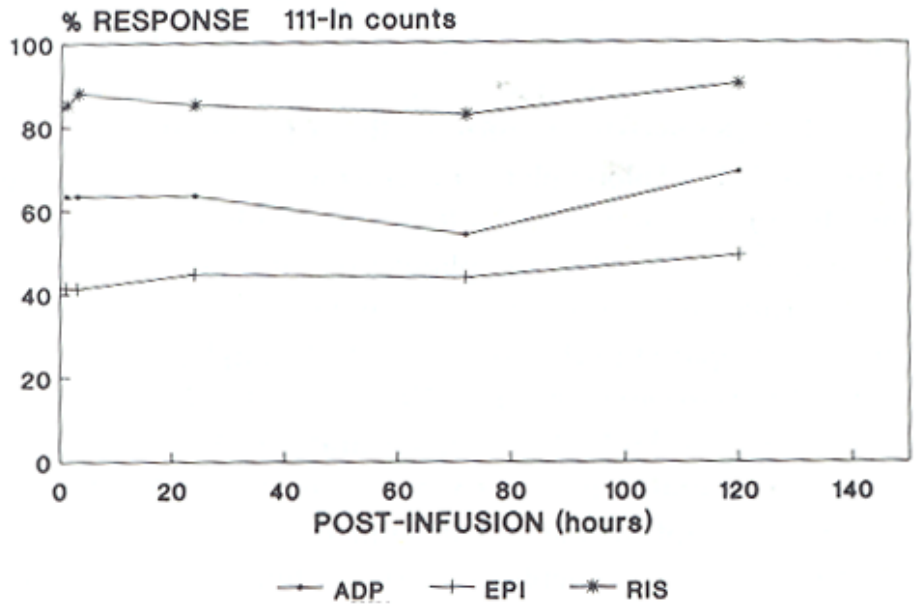
Post Transfusion Functionality of ^{111}In Platelets



^{111}In labeled fresh platelets as function of post-infusion time.

***N* = 12**

No significant difference over 5 Days in vivo

^{111}In Aggregation = Numerical Aggregation

BJH 80:539:1992

Double Label ^{111}In & ^{51}Cr In Vivo Studies

- Described a Double Label Method Identifying
  - Labeling issues relative to selective tracer uptake.
  - Technical issues relative to differential radionuclide counting.
  - Procedural issues relative to result acquisition/interpretation.
- Reviewed Some of the Physiological Observations Associated with PC Storage
  - Storage associated loss of in vivo efficacy.
  - Sites of storage damaged platelet uptake.
  - Chronological variation in platelet turnover.
- Proposed a Study Model to Allow Accurate Kinetic Analysis
  - Provided insight into P.C. functional recovery.
  - Suggested a driver to platelet senescence.